UNIVERSITI PUTRA MALAYSIA

EFFECTS OF TOPICAL APPLICATION OF EUPATORILUM ODORATUM, CHANNA STRIATUS, CENTELLA ASLAT ICA AND SILVER SULPHADIAZINE ON BURN WOUNDS IN AN ANIMAL MODEL

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By

NUR FAIZAH BT MUSTAFA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

July 2005

Dedicated with love to:

My parents, my in laws, my husband,my child,

my sisters and brothers and also my brother-in law

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

EFFECTS OF TOPICAL APPLICATION OF *EUPATORIUM ODORATUM*, *CHANNA STRIATUS*, *CENTELLA ASIATICA* AND SILVER SULPHADIAZINE ON BURN WOUNDS IN AN ANIMAL MODEL

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Faculty : Veterinary Medicine

The merit of attempting to treat burn wounds has long been appreciated since the last century, at least in the sense of providing a clean wound, avoiding formation of purulent and exudation as well as to enhance a granulation and reepithelization. It has been a special consideration in medical practice as burn denatures cellular protein, inhibits cellular metabolism hence secondary interference of local vascular supply.

Therefore, the present study was designed to investigate the effect of a crude methanolic extract of *Eupatorium odoratum, Channa striatus* and *Centella asiatica* on burn wound healing as these natural resources have been traditionally used in burn treatment. A standard reproducible of second

degree burn wounds was inflicted using a cylindrical stainless steel template (2.5 cm diameter) on 225 adult male Sprague Dawley weighing between 250 - 350g. The animals were divided into five groups with nine animals in each group, representing a control and experimental groups. Extracts of *Eupatorium odoratum, Channa striatus* and *Centella asiatica* with silver sulphadiazine, as a standard treatment were applied twice daily, except in the control group where wounds were left without any topical treatment.

The rats were closely monitored to assess any changes. The rats were euthanized at 3, 7, 14, 21 and 28 days post burned. The macroscopic appearance of burn wounds was evaluated and recorded. The percentage of wound contractions was measured. The burn sites were excised and subjected to water content assessment and biomechanical study. Apart from that, histological study was also performed qualitatively and quantitatively using a hematoxylin and eosin and Masson's trichrome staining.

Results obtained from this study revealed that from macroscopic study, *Eupatorium odoratum* showed advanced effect to minimize the progression of zone of stasis as compared to other groups. Quantitative evaluation of the number of inflammatory cells (polymorphonuclear leucocytes, and macrophages) from day 3 to day 28 in wounds treated with *Eupatorium odoratum* demonstrated significant fall in number of inflammatory cells (polymorphonuclear leucocytes, and macrophages) from day 3 to day 28, whereas the number of proliferative cells (fibroblasts and endothelial cells)

increased from day 7 to day 28. The *Eupatorium odoratum* also showed potential to preserve viable dermal tissue and induce a well-formed of angiogenesis with better organization as compared to other treatments. A semi quantitative wound scoring system used to evaluate the collagen bundles indicated that the *Eupatorium odoratum* treated burns demonstrated a better orientation of collagen as compared to other experimental groups as characterized by more densely packed fibres with thick bundles of well-aligned collagen and showed a basket-weave-like pattern with a more random structure. *Eupatorium odoratum* also promoted remodeling of collagen by synthesis of inter and intra-molecular protein crosslinking and thus produced a marked increased ($p<0.05$) in tensile strength as compared to other experimental groups. *Eupatorium odoratum* also consistently prevented burn edema as shown by reduction in wet to dry weight ratio of the burn site tissues. On the other hand, burn wounds treated with *Channa striatus* showed earlier re-epithelialization as early as 3 days post burned while *Eupatorium odoratum* treated burn wounds at day 7. The results also demonstrated that burn wounds treated with *Channa striatus* showed rapid cleansing of the wound with minimal scarring at day 28.

In conclusion, the present study showed that the *Eupatorium odoratum* was the most superior treatment agent for burn wounds followed by *Channa striatus* and then *Centella asiatica* in relation to macroscopic evaluation, histological findings, edema measurement and biomechanical property.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN SAPUAN *EUPATORIUM ODORATUM, CHANNA STRIATUS, CENTELLA ASIATICA* DAN SILVER SULPHADIAZIN KE ATAS LUKA TERBAKAR DENGAN MENGGUNAKAN HAIWAN SEBAGAI MODEL

Oleh

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Julai 2005

Pengerusi: **Md. Zuki Bin Abu Bakar@Zakaria, PhD**

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Semenjak abad yang lalu, usaha untuk merawat kecederaan terbakar telah mendapat perhatian yang luas, sekurang-kurangnya untuk menyediakan keadaan luka yang bersih, menghindarkan daripada berlakunya pernanahan dan eksudasi, malah ianya turut ditumpukan dalam usaha untuk membentuk semula proses granulasi dan pembinaan sel epithelium. Oleh kerana kebakaran mampu memusnahkan sel-sel protein, merencat berlakunya proses metabolisme sel dan menghalang pengaliran darah setempat secara sekunder, maka ia mendapat perhatian yang khusus di sektor perubatan.

Sehubungan dengan itu, satu kajian telah direkabentuk untuk melihat keberkesanan *Eupatorium odoratum, Channa striatus* dan *Centella asiatica* yang diekstrak secara mentah dengan menggunakan sebatian metanol terhadap luka terbakar memandangkan sumber asli ini telah digunakan secara tradisional untuk merawat luka terbakar. Luka terbakar ini telah dihasilkan secara seragam dengan menggunakan templat keluli tahan karat yang berbentuk bulat (berdiameter 2.5 cm) ke atas 225 ekor tikus jantan dewasa daripada spesis Sprague Dawley yang mempunyai berat diantara 250 - 300 g. Kesemua tikus ini telah dibahagikan kepada lima kumpulan dimana setiap kumpulan mengandungi 9 ekor tikus meliputi kumpulan tikus yang dirawat dan kumpulan tikus ujikaji terkawal. Ekstrak *Eupatorium odoratum, Channa striatus* dan *Centella asiatica* beserta krim silver sulphadiazin yang bertindak sebagai rawatan piawai diberikan secara sapuan kepada setiap kumpulan kecuali kumpulan tikus ujikaji terkawal yang tidak diberi sebarang rawatan.

Kesemua tikus telah dipantau secara dekat bagi melihat sebarang perubahan di bahagian luka. Tikus-tikus ini telah dimatikan pada hari ke 3, 7, 14, 21 dan 28 selepas luka. Pemeriksaan secara makroskopi telah dijalankan dan direkodkan secara bergambar dan peratusan kontraksi luka dikira. Bahagian tisu yang luka ini turut diambil untuk kajian kandungan air and sifat biomekanikal. Selain daripada itu, kajian histologi turut dijalankan secara kualitatif dan kuantitatif

dengan menggunakan pewarnaan hematoxylin dan eosin dan Masson's trichrome.

Keputusan yang dicapai daripada ujikaji ini telah mendedahkan secara makroskopi, bahawa *Eupatorium odoratum* terbukti paling berkesan untuk meminimumkan berlakunya penyebaran luka pada zon stasis jika dibandingkan dengan kumpulan yang lain. Ujian secara kuantitatif pula telah menunjukkan bahawa luka yang dirawat dengan *Eupatorium odoratum* berkecenderungan untuk menurunkan bilangan sel inflamasi (polimorfonuklear leukosit dan makrofaj) bermula daripada hari ke 3 hingga ke 28, malah didapati juga berkesan untuk meningkatkan bilangan sel proliferasi (sel fibroblas dan sel endotelial) daripada hari ke 7 hingga 28. Bertentangan dengan kumpulan rawatan jenis lain, ia juga berpotensi untuk memelihara sel-sel dermal yang hidup dan menggalakkan pertumbuhan dan struktur yang lebih baik terhadap sel-sel darah. Sistem pemarkahan secara separa kuantitatif selepas pewarnaan Masson's trichrome yang digunakan untuk mengevaluasi berkas kolagen telah menunjukkan bahawa kecederaan luka yang dirawat oleh *Eupatorium odoratum* memperlihatkan orientasi kolagen yang lebih sempurna dengan karakter yang lebih padat serta menunjukkan seolah-olah corak anyaman bakul yang tidak tersusun. Rawatan dengan *Eupatorium odoratum* juga menggalakkan proses pembentukan semula ke atas kolagen, dengan mensintesis silangpangkah secara inter dan intra-molekul yang seterusnya menghasilkan peningkatan yang

signifikan terhadap ujian regangan ($p<0.05$). Malahan, terbukti juga bahawa *Eupatorium odoratum* secara konsisten berkesan untuk menghalang berlakunya edema yang ditunjukkan dengan penurunan nisbah berat basah ke atas berat kering. Selain itu, ujian ini juga telah menunjukkan kecederaan luka yang dirawat dengan *Channa striatus* berupaya menghasilkan proses pembinaan semula epithelium sel seawal 3 hari selepas luka dikenakan berbanding dengan rawatan *Eupatorium odoratum* pada hari ke 7. Pada masa yang sama juga, rawatan dengan menggunakan ekstrak *Channa striatus* telah terbukti berupaya membersihkan luka dengan cepat dengan kadar parut yang minima pada hari ke 28.

Kesimpulannya, kajian ini telah menunjukkan bahawa ekstrak *Eupatorium odoratum* adalah bahan yang unggul dalam mengubati luka terbakar diikuti oleh *Channa striatus* dan kemudian *Centella asiatica* daripada segi pengevaluasian secara makroskopi, penemuan pada kajian histologi, pengiraan edema dan kandungan sifat mekanikal.

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I certify that an Examination Committee met on 13th July 2005 to conduct the final examination of Nur Faizah Mustafa on her Master of Science thesis entitled "Effects of Topical Application of *Eupatorium odoratum*, *Channa striatus*, *Centella asiatica* and *Silver sulphadiazine* on Burn Wounds in an Animal Model" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows :

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